

Amendments to the Specification

Please replace paragraph [0004] at page 1 with the following paragraph:

However, all of these known methods are extremely laborious. For example, Schneiderbauer extracts pine or spruce specimen with acetone at -70°C to remove polyphenolics. The pellet is then homogenized in the presence of 0.1% (v/v) ~~Triton~~ TRITON[®] X-100 (octyl phenol polyethoxylate), 15 mM DTT (dithiothreitol) and phenol. The homogenization process releases RNA, DNA, and proteins. Proteins are removed by phase separation in an organic extraction phase. Then, DNA is removed by centrifugation on a cesium chloride cushion.

Please replace paragraph [0014] at page 3 with the following paragraph:

The primary detergent may be any of the non-ionic detergents available, or in use: e.g., ~~Igepal~~ IGEPAL[®] (tergitol) (tert-octylphenoxy poly(oxyethylene) ethanol) (NP-40 replacement), ~~Tritons~~ TRITON[®]s, (~~Triton~~ TRITON[®] X-100 (octyl phenol polyethoxylate)), ~~Tween~~ TWEEN[®] 20 (polyoxyethylene sorbitan monolaurate) and like kind, etc., and is chosen for its ability to extract RNA without co-isolation of DNA. Preferably, non-ionic detergent is present at a concentration of about 0.1-4% by volume, more preferably at a concentration of about 0.5-3%, or about 1%-2%. A suitable non-ionic detergent is ~~Igepal~~ IGEPAL[®] (tergitol) (tert-octylphenoxy poly(oxyethylene) ethanol) at a concentration of 1% by volume.

Please replace paragraph [0028] at page 6 with the following paragraph:

Two formulations were used in the examples described below as preferred formulations. Other formulations are suitable generically or for specific plant tissues. Preferred formulations are the 40% 2-Mercaptoethanol Formulation and the 20% 2-Mercaptoethanol Formulation. These preferred formulations are listed below:

40% 2-Mercaptoethanol Formulation

1% ~~Igepal~~ IGEPAL[®] (tergitol)
100 mM EDTA
0.2% SDS
40% 2-mercaptoethanol
0.5% sodium azide

20% 2-Mercaptoethanol Formulation

1% ~~Igepal~~ IGEPAL[®] (tergitol)
100 mM EDTA
0.02% SDS
20% 2-mercaptoethanol
0.5% sodium azide

Please replace Table 1 at page 11 with the following table:

Table 1. RNA Yields from 100 mg of White Pine Spring Shoot					
% Igepal IGEPAL® (tergitol)	0.1 M EDTA in all assays, and Post Extraction NaCl, M Concentration	%SDS	mM DTT	RNA, µg	RNA Quality by Gel Analysis
1	0	0	0	19.0	Degraded
2	0	0	0	17.7	Degraded
4	0	0	0	16.9	Degraded
2	0	0	20	17.9	28S present
2	0	0.02	0	18.1	Degraded
2	0	0.02	20	22.8	28S present
2	0	0.1	0	26.2	Degraded
2	0	0.2	0	31.3	Degraded
4	0	0	20	22.4	28S present
4	0	0.02	0	24.8	Degraded
4	0	0.02	20	28.0	28S present
4	0	0.1	0	19.5	Degraded
4	0	0.2	0	33.0	Degraded
1	0	0.02	20	22.6	28S present
4	.25	0.02	20	27.2	28S present
4	.5	0.02	20	21.4	28S present
4	2.5	0.02	20	17.5	28S present

Please replace Table 2 at page 11 with the following table:

Table 2. RNA Yields from 100 mg of White Pine Spring Shoot					
% Igepal IGEPAL® (tergitol)	%SDS	mM DTT	% 2- Mercapto- ethanol	RNA, µg	RNA Quality by Gel Analysis: 28S Band Present?
1	0.02	20	0	27.9 28.8	Yes
1	0.2	100	0	34.2	Yes
4	0.02	20	0	34.4 26.5	Yes
4	0.02	100	0	35.2	Yes
1	0.02	0	0.4	31.7	Yes
1	0.2	0	4.0	50.4	Yes, Highest RNA Quality
4	0.02	0	0.4	30.8	Yes
4	0.2	0	4.0	12.2	yes

Please replace Table 4 at page 13 with the following table:

Table 4. Comparison of RNA Yields from 100 mg of Plant Needles or Leaves Using RNA Isolation Reagent vs. Other Methods				
Sample	RNA Isolation Reagent Chloroform extraction format µg RNA (A_{260/280})	RNA Isolation Reagent Cartridge Purification µg RNA (A_{260/280})	TRIzol µg RNA (A_{260/280})	RNeasy µg RNA (A_{260/280})
Blue spruce	16.0 ^a (2.07)	10.5 ^a (2.08)	0.8 (0.92)	4.0 (1.28)
Scrub pine	10.5 ^b (1.92)	17.7 ^b (1.99)	52.4 (1.07)	2.6 (1.21)
White pine	37.2 ^b (2.04)	12.8 ^b (1.78)	16.0 (1.11)	1.6 (1.26)
Juniper	18.0 ^a (1.49)	7.7 ^a (1.75)	14.4 (1.19)	3.0 (1.33)
Cedar	6.0 ^a (1.52)	8.3 ^a (1.65)	126.4 (1.23)	3.2 (1.10)
Holly	39.2 ^b (1.98)	22.6 ^b (1.89)	47.4 (2.01)	23.6 (2.11)
Hemlock	15.9 ^a (1.60)	16.5 ^a (1.76)	57.0 (1.35)	8.6 (1.07)

a 40% 2-mercaptoethanol/100 mM EDTA /1% ~~Igepal~~ IGEPAL[®] (tergitol)/0.2% SDS

b 20% 2-mercaptoethanol/100 mM EDTA /1% ~~Igepal~~ IGEPAL[®] (tergitol)/0.2% SDS

Please replace Table 6 at page 14 with the following table:

Table 6. RNA Yields (40% βME, 1% Igepal IGEPAL[®] (tergitol), 0.2% SDS)			
Plant Tissue	RNA Isolation Reagent μg RNA ($A_{260/280}$)	RNeasy μg RNA ($A_{260/280}$)	TRIzol μg RNA ($A_{260/280}$)
Arabidopsis leaves	37.8 (2.17)	23.8 (2.20)	32.8 (2.00)
Corn leaves	17.7 (2.13)	18.3 (2.16)	32.3 (1.95)
Rice leaves	7.0 (2.16)	14.7 (2.04)	21.5 (1.96)
Plum leaves	23.0 (1.92)	0.6 (1.28)	12.7* (1.13)
Tomato leaves	13.3 (1.99)	1.8 (0.96)	19.1* (1.44)
Tomato roots	7.8 (2.00)	5.3 (1.95)	7.5 (1.73)
Potato tuber	15.7 (1.98)	2.0 (1.25)	69.9* (1.46)

* Represent OD's, little to no RNA present by gel analysis.

Please replace Table 7 at page 15 with the following table:

Table 7. RNA Yields (%DNA Contamination):				
Plant	40% 2-ME with 1% Igepal <u>IGEPAL®</u> (tergitol) 0.2% SDS 0.1%CTAB RNA, µg	40% 2-ME with 1% Igepal <u>IGEPAL®</u> (tergitol) 0.5%CTAB RNA, µg	40% 2-ME 1% Igepal <u>IGEPAL®</u> (tergitol) 0.2% SDS RNA, µg	20% 2-ME no 1% Igepal <u>IGEPAL®</u> (tergitol) 0.2% SDS RNA, µg
Arabidopsis leaves	34.3 (5.5)	44.9 (2.1)	42.1 (2.8)	44.7 (4.4)
Corn leaves	13.3 (13.3)	19.4 (6.7)	17.5 (10.9)	24.9 (16.5)
Rice leaves	8.9 (14.4)	9.0 (7.3)	8.1 (16.3)	11.4 (32.3)
White pine, spring shoot	13.3 (1.7)	26.6 (1.4)	17.3 (1.4)	14.4 (4.5)

Please replace Table 8 at page 15 with the following table:

Table 8. RNA Yields (%DNA Contamination)						
Plant Leaf	40% βME 0.2% SDS 1% Igepal <u>IGEPAL®</u> (tergitol)	20% βME 0.2% SDS 1% Igepal <u>IGEPAL®</u> (tergitol)	20% βME 1% Igepal <u>IGEPAL®</u> (tergitol)	20% βME 0.02%SDS 1% Igepal <u>IGEPAL®</u> (tergitol)	20% βME 0.5%CTAB 1% Igepal <u>IGEPAL®</u> (tergitol)	20% βME .05%CTAB 1% Igepal <u>IGEPAL®</u> (tergitol)
Arabi- dopsis	37.8 (2.8)	52.6 (4.4)	55.0 (5.1)	54.0 (5.6)	42.8 (4.7)	53.6 (3.8)
Corn	15.1 (10.9)	24.9 (16.5)	29.1 (9.6)	37.8 (13.8)	21.9 (5.7)	30.0 (5.8)
Rice	7.9 (7.6)	19.0 (13.2)	38.3 (18.5)	17.0 (9.7)	14.3 (3.5)	33.5 (6.3)
Tomat o Tomato	15.8 (3.9)	11.3 (12.0)	12.8 (11.7)	17.3 (13.3)	13.7 (4.7)	20.6 (8.5)

Please replace Table 9 at page 15 with the following table:

Table 9. RNA Yields in μg from Popcorn Seeds (%DNA Contamination)			
Popcorn amount, g	40% βME 1% <u>Igepal IGEPAL[®]</u> (tergitol) 0.2% SDS	20% βME 1% <u>Igepal IGEPAL[®]</u> (tergitol) 0.02% SDS	20% βME 1% <u>Igepal IGEPAL[®]</u> (tergitol) 0.05% SDS
1.0	1.2 (17) 1.4 (71)	172.8 (6.6) 178.3 (5.7)	199.4 (9.1) 163.1 (10.9)

Please replace Table 10 at page 16 with the following table:

Table 10. RNA Yields (%DNA Contamination): Plant Reagent*		
Protocol Optimization		
Tissue	Salt**	RNA Yield, μg (%DNA)
Sugarbeet leaf, 1g	None	531.3 (4.8)
Sugarbeet leaf, 1g	2.5 M Am. Acetate	421.3 (5.6)
Popcorn seeds, 1g	None	0.0
Popcorn seeds, 1g	2.5 M Am. Acetate	227 (9.6)
Popcorn seeds, 5 g	1 M NaCl	1060.8 (6.4)
		1320.8 (5.9)
Potato tuber, 5g	1 M NaCl	922.5 (3.7)

*20% 2-mercaptoethanol, 1% Igepal IGEPAL[®] (tergitol), 0.02% SDS, 100 mM EDTA, 0.5% sodium azide formulation

**Salt added to the clarified RNA extract before chloroform addition.

Please replace Table 11 at page 16 with the following table:

Table 11. RNA Yields from 100 mg of 15-Day-Old Soybean Tissues (µg)*			
Tissue	RNA Isolation Reagent	RNeasy	TRIzol
Leaves	78.4	62.5	90.8
	86.6	53.3	110.2
Stems	39.5	33.4	32.1
	39.7	27.7	27.0
Roots	24.6	15.9	15.2
	19.3	15.0	12.8

*20% 2-mercaptoethanol, 1% ~~Igepal~~ IGEPAL[®] (tergitol), 0.02% SDS, 100 mM EDTA,
0.5% sodium azide formulation

Please replace Table 12 at page 17 with the following table:

Table 12. RNA Yields Using Plant Reagent*		
Sample	Amount, g	RNA Yield, mg
Arabidopsis whole plant	20	9.7
Tomato leaves	10	5.0
		3.9
Rice leaves	10	4.1
		4.7
		4.1
Corn leaves	30	9.5
Field corn seeds	40	8.0
Fungal mycelia	1	2.7

*20% 2-mercaptoethanol, 1% ~~Igepal~~ IGEPAL® (tergitol), 0.02% SDS, 100 mM EDTA, 0.5% sodium azide formulation

Please replace Table 13 at page 18 with the following table:

Table 13. RNA Yields from 100 mg of Plant Materials (%DNA Contamination)*				
Plant Material	Conifer Reagent RNA, µg (A_{260/280})	Qiagen RNeasy RNA, µg (A_{260/280})	TRIzol RNA, µg (A_{260/280})	Ambion/Plant Aid RNA, µg (A_{260/280})
Potato Tuber	21.9 (1.6) 31.5 (2.0)	1.1 (1.5) 0.7 (1.5)	5.8 (1.7) 18.4 (1.8)	0.0 0.0
Potato Leaves	62.9 (2.0) 93.8 (2.0)	46.0 (2.0) 38.0 (1.5)	26.6 (1.5) 50.2 (1.6)	3.0 (1.9) 12.0 (2.2)
Soybean Seeds	27.0 (2.0) 67.4 (2.0)	12.7 (2.0) 4.0 (2.2)	110 (1.7) 112 (1.7)	1.6 (1.8) 4.6 (1.2)
Soybean Leaves	135.8 (2.0) 114.5 (1.7)	44.1 (2.0) 43.4 (2.0)	109 (1.6) 33.4 (1.8)	0.8 (1.5) 2.5 (1.8)
White Pine Spring Shoot	21.8 (1.8) 18.3 (1.8)	0.5 (1.4) 2.5 (1.5)	9.3 (1.1) 10.4 (1.1)	0.9 (1.4) 0.8 (1.2)
Blue Spruce Needles	13.8 (1.8) 27.4 (1.8)	2.0 (1.4) 8.8 (1.0)	5.1 (1.4) 2.8 (1.2)	0.9 (1.2) 1.0 (1.2)
Tomato Leaves	42.0 (2.0) 179 (1.4)	10.6 (2.0) 5.5 (1.9)	22.0 (1.8) 25.0 (1.8)	1.9 (1.7) 9.4 (1.1)
Arabidopsis Whole Plant	148 (1.7) 52.0 (1.9)	25 (1.7) 30 (1.9)	36.0 (1.8) 18.0 (1.7)	3.9 (2.0) 3.2 (2.0)

*20% 2-mercaptoethanol, 1% Igepal IGEPAL® (tergitol), 0.02% SDS, 100 mM EDTA, 0.5% sodium azide formulation